

## CLAIMS

What is claimed is:

1. Isolated RNA of from about 21 to about 23 nucleotides that mediates RNA interference of an mRNA to which it corresponds.
- 5 2. Isolated RNA of claim 1 that comprises a terminal 3' hydroxyl group.
3. Isolated RNA of claim 1 which is chemically synthesized RNA or an analog of a naturally occurring RNA.
4. An analog of isolated RNA of claim 1, wherein the analog differs from the RNA of claim 1 by the addition, deletion, substitution or alteration of one or more  
10 nucleotides.
5. Isolated RNA of from about 21 to about 23 nucleotides that inactivates a corresponding gene by transcriptional silencing.
6. A soluble extract that mediates RNA interference.
7. The soluble extract of Claim 6, wherein the extract is derived from Drosophila  
15 embryos.
8. The soluble extract of Claim 7 wherein the extract is derived from syncytial blastoderm Drosophila embryos.
9. A method of producing RNA of from about 21 to about 23 nucleotides in length comprising:

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- (a) combining double-stranded RNA with a soluble extract that mediates RNA interference, thereby producing a combination; and
  - (b) maintaining the combination of a) under conditions in which the double-stranded RNA is processed to RNA of from about 21 to about 23 nucleotides in length.
10. The method of Claim 9, wherein the soluble extract is derived from syncytial blastoderm *Drosophila* embryos.
11. The method of Claim 9 further comprising isolating the RNA of from about 21 to about 23 nucleotides from the combination.
- 10 12. RNA of about 21 to about 23 nucleotides produced by the method of Claim 9.
13. A method of producing RNA of from about 21 to about 23 nucleotides in length that mediates RNA interference of mRNA of a gene to be degraded, comprising:
- (a) combining double-stranded RNA that corresponds to a sequence of the gene to be degraded with a soluble extract that mediates RNA interference, thereby producing a combination; and
  - (b) maintaining the combination of (a) under conditions under which the double-stranded RNA is processed to RNA of from about 21 to about 23 nucleotides that mediates RNA interference of the mRNA of the gene to be degraded, thereby producing RNA of from about 21 to about 23 nucleotides that mediates RNA interference of the mRNA.
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14. The method of Claim 13, wherein the soluble extract is derived from syncytial blastoderm *Drosophila* embryos.

15. The method of Claim 13 further comprising isolating RNA of from about 21 to about 23 nucleotides from the combination.
16. Isolated RNA of from about 21 to about 23 nucleotides produced by the method of Claim 15.
- 5 17. A method of mediating RNA interference of mRNA of a gene in a cell or organism comprising:
- (a) introducing RNA of from about 21 to about 23 nucleotides which targets the mRNA of the gene for degradation into the cell or organism;
- (b) maintaining the cell or organism produced in (a) under conditions under  
10 which degradation of the mRNA occurs, thereby mediating RNA interference of the mRNA of the gene in the cell or organism.
18. The method of Claim 17 wherein the RNA of (a) is a chemically synthesized RNA or an analog of naturally occurring RNA.
19. The method of Claim 17, wherein the gene encodes a cellular mRNA or a viral  
15 mRNA.
20. A method of mediating RNA interference of mRNA of a gene in a cell or organism in which RNA interference occurs, comprising:
- (a) combining double-stranded RNA that corresponds to a sequence of the gene with a soluble extract that mediates RNA interference, thereby  
20 producing a combination;
- (b) maintaining the combination produced in (a) under conditions under which the double- stranded RNA is processed to RNA of from about 21 to about 23 nucleotides, thereby producing RNA of from about 21 to about 23 nucleotides;

- (c) isolating RNA of from about 21 to about 23 nucleotides produced in (b);  
(d) introducing RNA isolated in (c) into the cell or organism; and  
(e) maintaining the cell or organism produced in (d) under conditions under which degradation of mRNA of the gene occurs, thereby mediating RNA interference of the mRNA of the gene in the cell or organism.

21. The method of Claim 20, wherein the soluble extract is derived from syncytial blastoderm *Drosophila* embryos.
22. The method of Claim 20, wherein the RNA is isolated using gel electrophoresis.
23. A method of mediating RNA interference of mRNA of a gene in a cell or organism in which RNA interference occurs, comprising: (a) introducing into the cell or organism RNA of from about 21 to about 23 nucleotides that mediates RNA interference of mRNA of the gene, thereby producing a cell or organism that contains the RNA and (b) maintaining the cell or organism that contains the RNA under conditions under which RNA interference occurs, thereby mediating RNA interference of mRNA of the gene in the cell or organism.
24. The method of claim 23, wherein the RNA of from about 21 to about 23 nucleotides is chemically synthesized RNA or an analog of RNA that mediates RNA interference.
25. The method of Claim 23, wherein the gene encodes a cellular mRNA or a viral mRNA.
26. A knockdown cell or organism generated by the method of claim 23.

27. The knockdown cell or organism of claim 26, wherein the cell or organism mimics a disease.
28. A method of examining the function of a gene in a cell or organism comprising:
- 5 (a) introducing RNA of from about 21 to about 23 nucleotides that targets mRNA of the gene for degradation into the cell or organism, thereby producing a test cell or test organism;
- (b) maintaining the test cell or test organism under conditions under which degradation of mRNA of the gene occurs, thereby producing a test cell or test organism in which mRNA of the gene is degraded; and
- 10 (c) observing the phenotype of the test cell or test organism produced in (b) and, optionally, comparing the phenotype observed to that of an appropriate control cell or control organism, thereby providing information about the function of the gene.
29. The method of Claim 28 wherein the RNA introduced in (a) is chemically synthesized or an analog of RNA that mediates RNA interference.
- 15 30. A method of examining the function of a gene in a cell or organism comprising
- (a) combining double-stranded RNA that corresponds to a sequence of the gene with a soluble extract that mediates RNA interference, thereby producing a combination;
- 20 (b) maintaining the combination produced in (a) under conditions under which the double- stranded RNA is processed to RNA of about 21 to about 23 nucleotides, whereby RNA of about 21 to about 23 nucleotides is produced;
- (c) isolating RNA of about 21 to about 23 nucleotides produced in (b);
- 25 (d) introducing the RNA isolated in (c) into the cell or organism, thereby producing a test cell or test organism;

- (e) maintaining the test cell or test organism under conditions under which degradation of mRNA of the gene occurs, thereby producing a test cell or test organism in which mRNA of the gene is degraded; and
- (f) observing the phenotype of the test cell or test organism produced in (e) and, optionally, comparing the phenotype observed to that of an appropriate control, thereby providing information about the function of the gene.
31. The method of claim 30, wherein the RNA comprises a terminal 3' hydroxyl group.
32. The method of claim 30, wherein the soluble extract is derived from syncytial blastoderm *Drosophila* embryos.
33. The method of claim 30, wherein the RNA is isolated using gel electrophoresis.
34. A composition comprising biochemical components of a *Drosophila* cell that process dsRNA to RNA of about 21 to about 23 nucleotides and a suitable carrier.
35. A composition comprising biochemical components of a cell that target mRNA of a gene to be degraded by RNA of about 21 to about 23 nucleotides.
36. A method of treating a disease or condition associated with the presence of a protein in an individual comprising administering to the individual RNA of from about 21 to about 23 nucleotides that targets the mRNA of the protein for degradation.

37. The method of claim 36 wherein RNA of from about 21 to about 23 nucleotides is chemically synthesized or an analog of RNA that mediates RNA interference.
38. A method of assessing whether an agent acts on a gene product comprising:
- (a) introducing RNA of from about 21 to about 23 nucleotides which targets the mRNA of the gene for degradation into a cell or organism;
  - (b) maintaining the cell or organism of (a) under conditions in which degradation of the mRNA occurs,
  - (c) introducing the agent into the cell or organism of (b); and
  - (d) determining whether the agent has an effect on the cell or organism, wherein if the agent has no effect on the cell or organism then the agent acts on the gene product or on a biological pathway that involves the gene product.
39. The method of claim 38, wherein the RNA of from about 21 to about 23 nucleotides is chemically synthesized or an analog of RNA that mediates RNA interference.
40. A method of assessing whether a gene product is a suitable target for drug discovery comprising:
- (a) introducing RNA of from about 21 to about 23 nucleotides which targets the mRNA of the gene for degradation into a cell or organism;
  - (b) maintaining the cell or organism of (a) under conditions in which degradation of the mRNA occurs resulting in decreased expression of the gene; and
  - (c) determining the effect of the decreased expression of the gene on the cell or organism, wherein if decreased expression has an effect, then the gene product is a target for drug discovery.

41. The method of claim 40, wherein the RNA of from about 21 to about 23 nucleotides is synthetic RNA or an analog of RNA that mediates RNA interference.
42. A gene identified by the sequencing of endogenous 21 to 23 nucleotide RNA molecules that mediate RNA interference.
43. A pharmaceutical composition comprising RNA of from about 21 to about 23 nucleotides that mediates RNA interference and an appropriate carrier.
44. A method of producing knockdown cells, comprising introducing into cells in which a gene is to be knocked down RNA of about 21 to about 23 nt that targets the mRNA corresponding to the gene and maintaining the resulting cells under conditions under which RNAi occurs, resulting in degradation of the mRNA of the gene, thereby producing knockdown cells.
45. The method of claim 44, wherein the RNA of about 21 to about 23 nucleotides is synthetic RNA or an analog of RNA that mediates RNA interference.
46. A method of identifying target sites within mRNA that are efficiently cleaved by the RNAi process, comprising combining dsRNA corresponding to a sequence of a gene to be degraded, labeled mRNA corresponding to the gene and a soluble extract that mediates RNA interference, thereby producing a combination; maintaining the combination under conditions under which the dsRNA is degraded and identifying sites in the mRNA that are efficiently cleaved.
47. A method of identifying 21-23 nt RNAs that efficiently mediate RNAi, wherein said 21-23 nt RNAs span the target sites identified within the mRNA by the method of claim 46.



48. RNA of claim 16, isolated using gel electrophoresis.
49. RNA of claim 16, isolated using non-denaturing methods.
50. RNA of claim 16, isolated using non-denaturing column chromatography.

ADD A1

ADD B1

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